

**On the Inhibition of Chymotrypsin by Ovomuroid**

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*From the Eastern Regional Research Laboratory,<sup>1</sup> Philadelphia 18, Pennsylvania*

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A study has been made of the inhibition of chymotrypsin by ovomucoid. Marked inhibition is obtained only at high ovomucoid to chymotrypsin ratios. Electrophoretic and ultracentrifugal experiments show that this inhibition is accompanied by complex formation between the two proteins.

### INTRODUCTION

Ovomucoid present in egg white has been shown to be an effective inhibitor of trypsin (1, 2). Furthermore, we have observed that the residual chymotryptic activity often found in trypsin preparations also disappeared on inhibition of trypsin by ovomucoid. The purpose of the present communication is to present evidence that, under certain conditions, in addition to inhibiting trypsin, ovomucoid can also inhibit pure chymotrypsin by complexing with it.

### EXPERIMENTAL

Throughout the experiments, Worthington<sup>2</sup> crystallized chymotrypsin and ovomucoid were used.

The chymotrypsin assay was carried out according to Schwert *et al.* (3) using 10 ml. of a 0.025 *M* solution of *N*-acetyl-L-tyrosine ethyl ester as a substrate at 25°C. In all assays, aliquots containing 25  $\mu$ g. chymotrypsin were used. The degree of inhibition was calculated from the linear portion of the reaction rate curve.

For the enzymic assays and sedimentation and electrophoresis experiments, 10 g./l. chymotrypsin and ovomucoid solutions were prepared by dissolving the proteins in a pH 7.0 phosphate buffer of 0.1 ionic strength. The solutions at various ratios were prepared at 4°C. immediately before the runs.

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<sup>2</sup> Mention of a specific commercial product does not constitute an endorsement by the U. S. Department of Agriculture.

The ultracentrifugal experiments were carried out in monochlorotrifluoroethylene polymer (Kel-F) cells in a Spinco model E analytical ultracentrifuge at 25°C. at 59,780 r.p.m. The electrophoresis runs were performed in a Spinco model H electrophoresis-diffusion apparatus at a field strength of 4.7 v./cm. The length of the runs was 180 min. Sedimentation constants and mobilities were measured with the help of a microcomparator. Area analysis was carried out planimetrically on enlarged projected tracings.

### RESULTS AND DISCUSSION

The degree of enzyme inhibition at various ratios of ovomucoid to chymotrypsin is presented in Fig. 1. The inhibition curve is S-shaped and shows that marked inhibition is present only in the region of high ovomucoid concentration.

In order to determine whether the inhibition is accompanied by molecular complex formation between chymotrypsin and ovomucoid, ultracentrifugal and electrophoretic measurements were carried out on mixtures of the two proteins at different ratios.

In the sedimentation experiments, the individual protein patterns had only one peak with  $s_{20,w}$  values of  $2.5 \times 10^{-13}$  for a 10 g./l. ovomucoid solution and  $2.6 \times 10^{-13}$  for chymotrypsin solutions, 1.7 and 10 g./l. in concentration. In the case of the mixtures, there appeared an additional more rapidly sedimenting peak with  $s_{20,w} = 6.3 \times 10^{-13}$ . This "component" accounted for 4-7% of the total area under the ultracentrifugal patterns and varied little, within the limit of accuracy of area measurements, as the ovo-

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mucoid to chymotrypsin ratio increased from 1:1 to 1:20. The presence of the rapidly sedimenting component in the mixtures and its absence in the run on the 10 g./l. solution of chymotrypsin shows that the 6.3 *S* peak is not just a chymotrypsin aggregate formed in the presence of high total protein concentration, but that it reflects complexing when the two proteins are present together in solution. This is further supported by a sedimentation run in which the ovomucoid had been replaced by  $\beta$ -lactoglobulin. In this last run, only one peak with  $s_{20,w} = 2.6 \times 10^{-13}$  was obtained. There was no evidence for the presence of any heavier material.

The presence of ovomucoid-chymotrypsin complexing has been further demonstrated in electrophoretic experiments. Tracings of the descending limbs of the electrophoretic patterns are presented in Fig. 2. Pure chymotrypsin gives only one peak (dotted line for run on 1.7 g./l. solution) with a mobility of  $-0.35 \times 10^{-5}$  sq. cm./sec. v. Ovomucoid (solid line) has a main peak with a mobility of  $-3.5 \times 10^{-5}$  and a small peak (about 3% of total area) with a mobility of  $-1.3 \times 10^{-5}$ . The main peak displays incipient resolution into the several known components of ovomucoid (4, 5). In the case of the mixtures, four "components" appear in the patterns, two having mobilities of  $-0.35$  and  $-3.3$ , which correspond to chymotrypsin and ovomucoid, and two new ones with mobilities of  $-1.0 \times 10^{-5}$  and  $-2.1 \times 10^{-5}$ . In the figure, the patterns of the 1:5 and 1:10 chymotrypsin-ovomucoid mixtures are shown by the dot-dash and the dashed lines, respectively. Comparison of the pure chymotrypsin pattern with that of the 1:5 mixture reveals a significant decrease in area under the chymotrypsin peak when ovomucoid is present, although the total amount of chymotrypsin is identical in the two solutions. This decrease in the chymotrypsin peak is accompanied by the formation of two new boundaries having mobilities intermediate between those of the two individual proteins. This would be expected of complexes between two proteins. The same type of behavior is also observed in the other mixtures studied, as shown in Fig. 2 for the 1:10 solution. The appearance of two new peaks

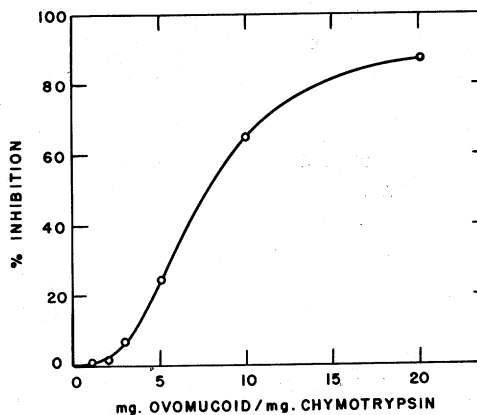


FIG. 1. Inhibition of chymotrypsin by ovomucoid.

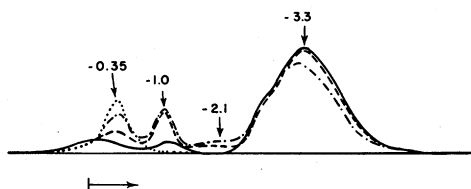


FIG. 2. Descending patterns of electrophoretic pictures. Dotted line: pure chymotrypsin (1.7 g./l.); solid line: pure ovomucoid (10.0 g./l.); dot-dash line: 1:5 chymotrypsin-ovomucoid mixture (10.0 g./l.); dashed line: 1:10 chymotrypsin-ovomucoid mixture (10.0 g./l.). 180 min. at 4.7 v./cm. Numbers represent mobilities of individual peaks.

( $-1.0$  and  $-2.1 \mu$ ) during interaction indicates that the complexing pattern is not a simple one and that possibly more than one complex may be involved.

The ultracentrifugal and electrophoretic evidence presented above seem to establish, in a qualitative way at least, that the inhibition of chymotrypsin by ovomucoid is accompanied by the formation of complexes between the two proteins, as is in the case of trypsin inhibition by ovomucoid (6). Since in interacting systems in rapid equilibrium the areas under the moving boundaries and "reaction boundaries" (7) are a complicated function of the stoichiometry, rates of migration, and rates of re-equilibration (8, 9), no attempt at quantitative analysis of the present data can be made. The enzyme inhibition data, however, would indicate that the interaction is weak and that the dissociation constants of the complexes are high. As

a result, marked inhibition is obtained only at high ovomucoid to chymotrypsin ratios. Furthermore, in comparing inhibition data with complexing, one should bear in mind the findings of Bier *et al.* (6) that in the trypsin-ovomucoid case, several complexes are formed which vary in degree and nature of inhibition.

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